IN VITRO CYTOTOXICITY STUDY OF PLANT *ALOE VERA* AGAINST CANCER CELL LINES

A dissertation submitted to

THE TAMIL NADU Dr. M.G.R. MEDICAL UNIVERSITY, CHENNAI

In partial fulfillment of the requirement for
The award of the degree of

MASTER OF PHARMACY
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Submitted by

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Under the Guidance of

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TAMILNADU, ERODE - 52.

MARCH – 2009

CERTIFICATE

This is to certify that the work embodied in this thesis entitled, ***"In vitro* Cytotoxicity Study *of* Plant *Aloe vera* Against Cancer Cell Lines"** submitted to The Tamil Nadu Dr. M.G.R. Medical University, Chennai, was carried by **Mr. Atul Nishant Chandu (Reg. No. 26073822)**, Department of Pharmaceutical Bio-technology, Nandha College of Pharmacy, Erode-52 for the partial fulfillment for the degree of **MASTER OF PHARMACY** in Pharmaceutical Bio-technology under our supervision.

This work is original and has not been submitted in part or full for any other degree or diploma of this or any other university.

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Declaration

I declare that I have carried out the work presented in this thesis entailed**, "*In vitro* Cytotoxicity study of plant *Aloe vera* against cancer cell lines"** in the Department of Pharmaceutical Biotechnology, Nandha College of Pharmacy Erodde-52 under the guidance of **Mr. K. Kamalakannan, M.Pharm, Assistant Professor**, Department of Pharmaceutical Biotechnology, Nandha College of Pharmacy, Erode52.

This work is original and has not been previously submitted in plant full for the award of any other degree, diploma, associate ship fellowship or any other similar litter of any other university.

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List of abbreviations and symbols

Bd. Wt.	Body weight
Conc.	Concentration
DDW	Double distilled water
Hr.	Hour
Min.	Minutes
AP	Apoptosis
NC	Normal cells
VC	Viable cells
TC	Total cells
rpm	Rotation per minutes
%	Percent
µg	Microgram
g	Gram
LAF	Laminar air flow
Mg	Miligram
Kg	Kilogram
w/v	Weight per unit volume
Dox	Doxorubicin
Fig	Figure
Ext.	Extract
AV	*Aloe vera*
PBS	Phosphate buffer solution

EMEM	Eagle's minimum essential medium
FCS	fetal calf serum
amt	Amount

1. Cancer

Cancer is a disease characterized by uncontrolled multiplication and spread of abnormal forms of the body's own cells. It is one of the major causes of death in the developed nations. One in three people will be diagnosed with cancer during their lifetime, and in 2001 (for example) 270000 new cases were reported in the United Kingdom. Cancer is also responsible for approximately one-quarter of all deaths in the United Kingdom, with lung and bowel cancer comprising the largest category, closely followed by breast and prostate cancer. At first sight, incidence figures for the past 100 years or so give the impression that the disease is increasing in developed countries, but cancer is largely a disease of later life, and with advances in public health and medical science many more people now live to an age where they are more liable to contract cancer.[1]

The ancient origin of the term cancer derives from the Latin word “crab”, cancer- presumably because a cancer adheres to any part that it seizes upon in obstinate manner like a crab. Two characteristics features define a cancer cell growth not regulated by external signals (i.e. autonomous) and the capacity to invade tissue and metastasize and colonized distant sites.

The first of these features, the uncontrolled growth of abnormal cell is a property of neoplasms or new growth. A neoplasm may be begin or malignant. If invasion, the 2nd cardinal feature of cancer is present, the neoplasm is malignant. Cancer is a synonym for malignant neoplasm. Cells that have undergone neoplastic transformation usually express cell surface antigens that may be of normal fetal type, may display other signs of apparent immaturity and

may exhibit qualitative or quantitative chromosomal abnormalities, including various translocations and the appearance of amplified gene sequences.

Cancer refers to the hyper proliferation of cells that have lost the ability to be controlled by normal cell signals. Cancer cells have the ability to proliferate independent of their environment and are capable of metastasizing, or colonizing other tissues in the body.

There are three basic characteristics of early cancer cells. The first is that they have lost the ability to undergo apoptosis, or programmed cell death. Cells that have suffered irreparable DNA damage activate specific proteases and nucleases that destroy the proteins and DNA of the cell, thereby effectively limiting the spread of potentially deleterious mutations. Cancer cells have often obtained mutations in genes involved in regulating this pathway.

Secondly, cancer cells have lost the ability to stop dividing. Normal cells require extracellular signals, such as growth factors, in order to activate pro-growth pathways. This need for extracellular stimulation is one means of regulating cell growth that cancer cells manage to bypass. This means that cancer cells proceed through the cell cycle and continue to divide indefinitely.

Thirdly, cancer cells often have abnormal telomerase activity. Telomeres are the specialized regions on the ends of chromosomes that form t-loops - these protect the ends and distinguish double-stranded DNA breaks from normal ends. Telomerase is an enzyme that acts to lengthen the telomeres on aging chromosomes in certain cell types. A portion of the telomere is

lost during each DNA replication. When telomeres are shortened to the point that they cannot form t-loops, cells undergo senescence, where the cell remains in an undividing state, or apoptosis. Cancer cells, which are continually dividing, often express telomerase or have increased telomerase activity in order to circumvent the telomere problem. These three basic characteristics are the premise for malignancy and enable the accumulation of more genetic and chromosomal abnormalities, which further lead to increased malignant and metastatic phenotypes.

There are several different kinds of cancer but they all essentially fall into three basic categories: carcinomas, sarcomas and leukemia/lymphomas. Both carcinomas and sarcomas are solid tumors, or tumors consisting of a dense collection of cancer cells that has its own blood supply.

Carcinomas are solid malignant lesions originating in the epithelial tissue. These are often found in glands or the lining of various organs. Examples of carcinomas would be breast cancer and prostate cancer, and occur most frequently in older patients. Sarcomas are solid tumours of the connective tissue, such as bone and muscle. There is a higher incidence of sarcoma in younger patients than in older ones. Finally, leukemia and lymphoma are malignancies originating in immune or haematopoeitic cells and can be found in patients representing a wide age range. These cancers are commonly referred to as disseminated cancers as they do not form masses of cells, but instead are found throughout the vascular system.

1.1 The Special Characteristics of Cancer Cells

Cancer cells manifest, to varying degrees, four characteristics that distinguish them from normal cells. These are:

- Uncontrolled proliferation.
- Differentiation and loss of function.
- Invasiveness.
- Metastasis.

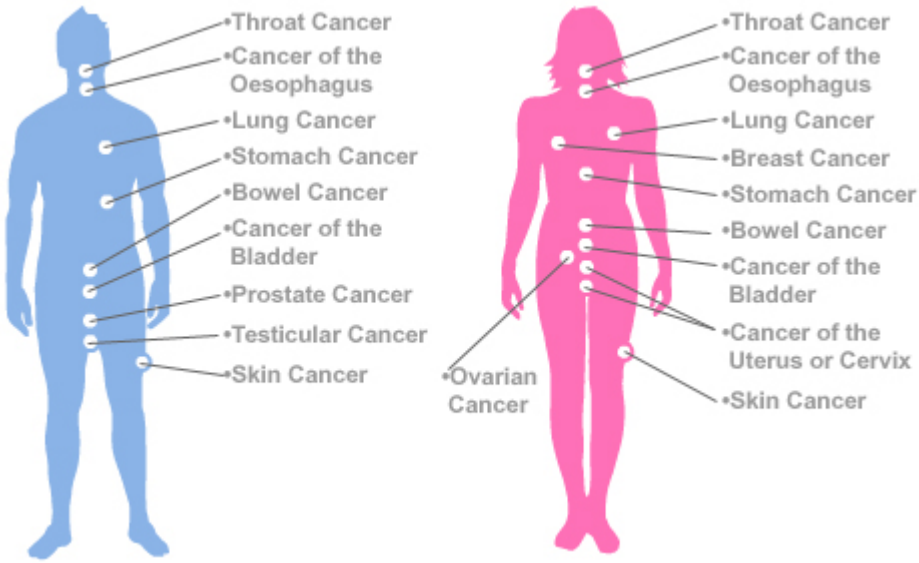
1.2 Causes of Cancer

The incidence, geographic distribution, and behavior of specific types of cancer are related to multiple factors, including sex, age, race, genetic predisposition, and exposure to environmental carcinogens. Out of these factors, environmental exposure is probably most important. Exposure to ionizing radiation has been well established to be a significant risk factor for a number of cancers, including acute leukemia, thyroid cancer, breast cancer, lung cancer, soft tissue sarcoma, and basal cell skin cancers. Chemical carcinogens (particularly those in tobacco smoke) as well as azo dyes, aflatoxins, asbestos, benzene, and radon have been clearly implicated in cancer induction in humans and animals. Viruses have been implicated as the etiologic agents of several human cancers. Expression of virus-induced neoplasia probably also depends on additional host and environmental factors that modulate the transformation process.

1.3 Types of Cancer

Pathological classification:

- Carcinoma : Malignant tumors that arises from epithelial cell.
- Melanoma : Cancerous growth of melanocytes, skin epithelial cell that produce the pigment melanin.
- Sarcoma : Cancer arising from muscle cell or connective tissue.
- Osteogenic Sarcoma : The most frequent type of childhood cancer Destroys normal bone tissue.
- Leukemia : It is a cancer of blood forming organs characterized By rapid growth of abnormal leucocytes.



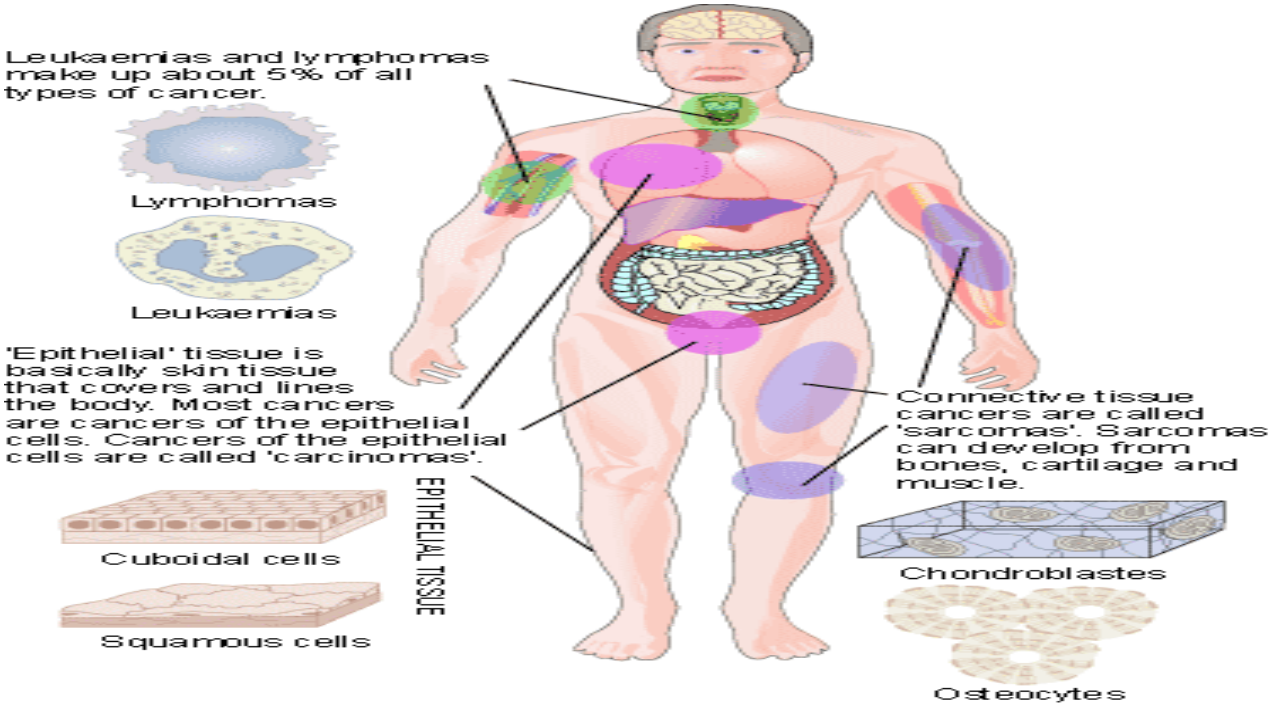
1.4 Melanoma Cancer

Melanoma is a form of skin cancer that arises from melanocytes - the cells that produce pigment. Melanoma may begin in association with a mole. Melanocytes produce a pigment called melanin that gives the skin its color and protects it from sun damage. Darker skin has more melanin and more protection. Melanocytes often cluster together and form moles (nevi). Most moles are benign, but some may go on to become malignant melanomas.

1.5 Types of Melanoma [5]

Melanoma, acral-lentiginous:

One of the four clinical types of malignant melanoma is the most common type in non whites but uncommon in whites. It starts as an irregular enlarging black flat spot (macule), most often on the palm and sole, less often on a mucosal surface, such as the vulva or vagina



.**Melanoma, lentigo maligna:**

One of the four clinical types of malignant melanoma and the slowest growing one. It typically begins as a patch of mottled pigmentation that is dark brown, tan, or black on sun-exposed skin, such as on the face.

Melanoma, nodular

One of the four clinical types of malignant melanoma, typically presents as a raised, distinct, bluish-black tumor that may be encircled by particularly pale skin, most often in middle-aged or older adults.

Melanoma, superficial spreading:

One of the four clinical types of malignant melanoma, the most common type in whites, typically presents as a raised, irregular, colored area that starts in a mole-like shape but spreads across the skin.

Stages of Melanoma Progression:

In situ melanoma:

The earliest form of melanoma, it is small and does not extend beneath the surface of the skin.

Stage I melanoma:

Tumors are less than 2mm in thickness, found in the outer layer of the skin (epidermis) and/or the upper part of the inner layer of the skin (dermis).

Stage II melanoma

The carcinoma spreads to the lower part of the inner layer of the skin (dermis) but not into the tissue below. It can spread into fat tissue as well.

Stage III melanoma

The carcinoma spreads to near by lymph nodes.

Stage IV melanoma

The carcinoma spreads beyond the skin to distant sites or organs. Melanoma can also start in the mucous membranes of the mouth, anus and vagina, in the eye or other places in the body where melanocytes are found.

1.6 Sarcoma

A sarcoma (from the Greek 'sarx' meaning "flesh") is a cancer of the connective tissue (bone, cartilage, fat) resulting in mesoderm proliferation.

This is in contrast to carcinomas, which are of epithelial origin (breast, colon, pancreas, and others). However, due to an evolving understanding of tissue origin, the term "sarcoma" is sometimes applied to tumors now known to arise from epithelial tissue. The term soft tissue sarcoma is used to describe tumors of soft tissue, which includes elements that are in connective tissue, but not derived from it (such as muscles and blood vessels).

Classification

Tissue: Sarcomas are given a number of different names, based on the type of tissue from which they arise. For example, osteosarcoma arises from bone, chondrosarcoma arises from cartilage, and leiomyosarcoma arises from smooth muscle. Sarcomas strike people in all age

ranges, but they are very rare, accounting for only 1% of all cases of cancer. Soft tissue sarcomas, such as leiomyosarcoma, chondrosarcoma, and gastrointestinal stromal tumor (GIST), are more common in adults than in children. GIST is the most common form of sarcoma, with approximately 3000-3500 cases per year in the United States.This should be compared with breast cancer, with approximately 200,000 cases per year in North America. Bone sarcomas, such as osteosarcoma and Ewing's sarcoma, are more common in children than in adults. These tumors most commonly strike adolescents and young adults between the ages of 12 and 25.

Grade: In addition to being named based on the tissue of origin, sarcomas are also assigned a grade, such as low grade or high grade. Low grade sarcomas are usually treated surgically, although sometimes radiation therapy or chemotherapy is used. High grade sarcomas are more frequently treated with chemotherapy. Since these tumors are more likely to undergo metastasis (spreading to distant sites), these tumors are treated more aggressively. Childhood sarcomas are almost always treated with a combination of surgery and chemotherapy, and radiation is frequently used as well. The recognition that childhood sarcomas are sensitive to chemotherapy has dramatically improved the survival of patients. For example, in the era before chemotherapy, long term survival for patients with localized osteosarcoma was only approximately 20%, but now has risen to 60-70%.

Fibrosarcoma

Fibrosarcoma (fibroblastic sarcoma) is a malignant tumor derived from fibrous connective tissue and characterized by immature proliferating fibroblasts or undifferentiated anaplastic spindle cells.

Pathology

The tumor may present different degrees of differentiation: low grade (differentiated), intermediate malignancy and high malignancy (anaplastic). Depending on this differentiation, tumor cells may resemble mature fibroblasts (spindle-shaped), secreting collagen, with rare mitoses. These cells are arranged in short fascicles which split and merge, giving the appearance

of "fish bone". Poorly differentiated tumors consist in more atypical cells, pleomorphic, giant cells, multinucleated, numerous atypical mitoses and reduced collagen production. Presence of immature blood vessels (sarcomatous vessels lacking endothelial cells) favors the bloodstream metastasizing

1.7 How Does Cancer Arise

Cancer arises most commonly in older patients but can occur at any age. Different cancers are more prevalent in certain age groups and are rarely found in people outside that age range. An example would be prostate cancer, where it is very uncommon for a man under the age of 40 to be diagnosed with the disease. It makes sense that cancer occurs predominantly in the elderly population, as cancer is the result of accumulated genetic mutations. As individual ages they are more likely to have been exposed to chemicals, radiation and other events that cause DNA damage. As a result, these individuals are more likely to have mutations in genes allowing for cancerous behaviors.

As mentioned above, characteristics like the ability to evade apoptosis; unregulated cell division and increased telomerase activity are all the results of genetic mutations that can lead to cancer. These mutations commonly occur in what are called proto-oncogenes and tumour suppressor genes. Proto-oncogenes are genes coding for proteins involved in cell cycle progression and growth signalling; when mutated these genes are called oncogenes (see Figure 2). Oncogenes are autosomal dominant, meaning that only one mutated allele is necessary for cancerous behavior to develop. Several oncogenes have been identified in many types of cancers, including myc, the bcl family of genes and ras.

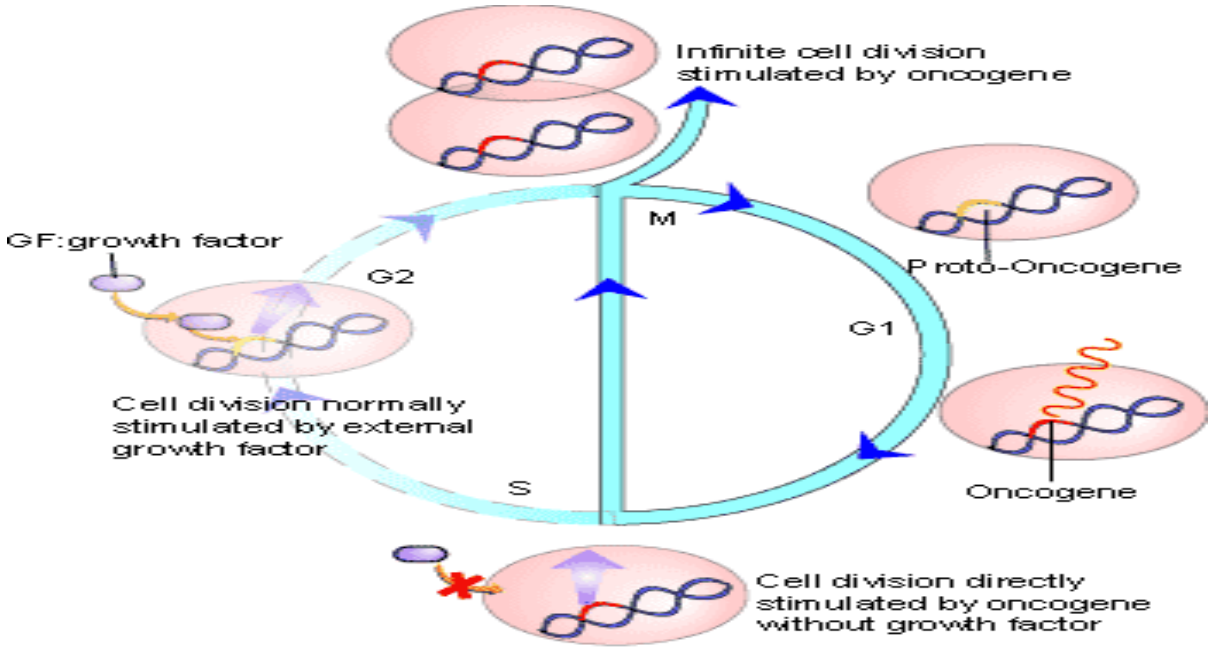


Figure 2. Oncogenes are mutated forms of normal cellular genes involved in growth signalling pathways (proto-oncogenes). When these genes become mutated the cell does not require the presence of pro-growth signals (e.g. growth factors) in order to undergo cell division.

In contrast, tumour suppressor genes are often involved in regulating apoptosis (see Figure 3). Cells that have sustained damage to their DNA apoptose under the control of tumour suppressors; when both alleles are mutated to be non-functional cells fail to enter the apoptotic pathway. These cells continue to divide and are not subjected to further DNA repair attempts, resulting in increasing genomic instability and further mutations. These mutations often occur in other oncogenes and tumour suppressor genes, conferring more cancerous behaviors. If either of these kinds of mutations exists or occurs in someone then they are at a higher risk of developing cancer.

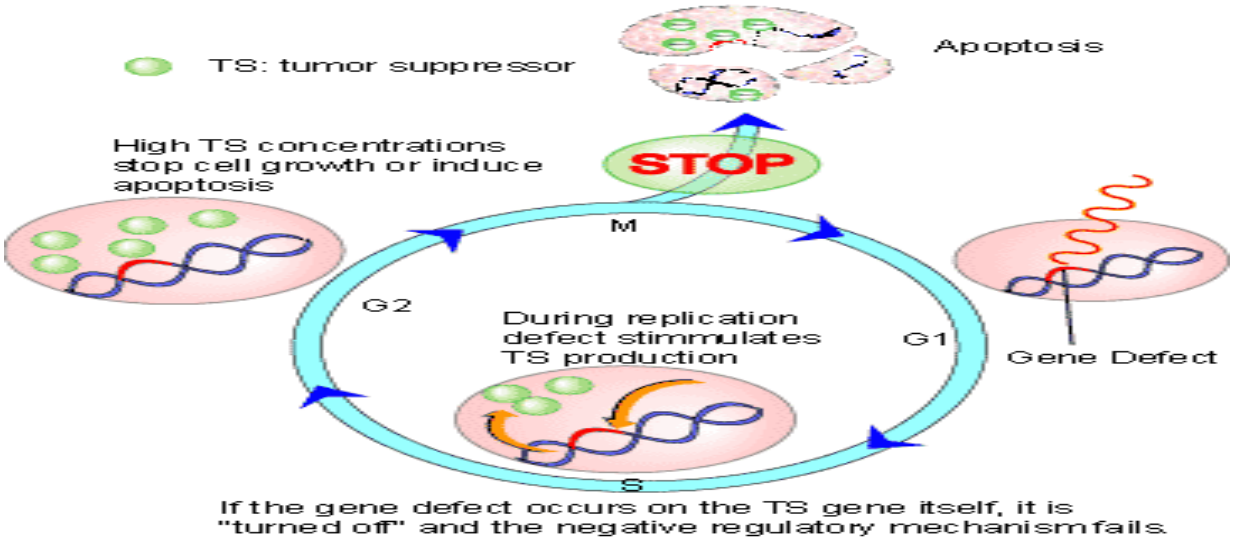
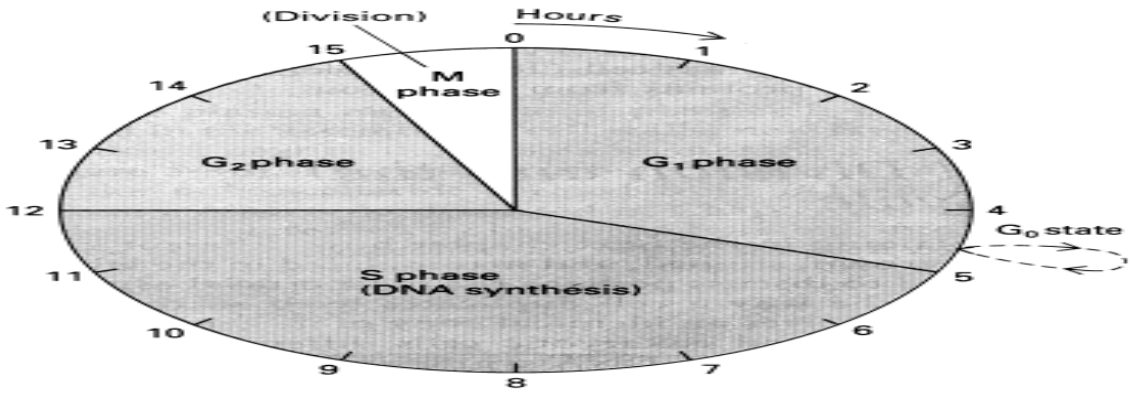


Figure 3. Tumour suppressor genes are genes often involved in the apoptotic pathway. Normally tumour suppressors detect breaks or defects in the DNA - if present in low concentrations these proteins will pause the cell cycle and active DNA repair mechanisms. If present in high concentrations, tumour suppressors shut down the cell cycle or cause apoptosis. When these genes are mutated to be dysfunctional then the cell does not undergo either of these events.

It is important to note that cancer results from the clonal expansion of one progenitor cell, or "mother" cell (the one that first obtained a mutation allowing for a "cancerous" phenotype). This means that all cells in a tumour should in fact be genetically identical to the parent cell and exhibit the same mutations, however, because mutations occur at a much higher rate in tumour cells, daughter cells may be more tumourogenic than the parent [4]. All that is required for cancer to develop is one cell among millions that is capable of developing independent of regulatory signals.

1.8 Importance of Cell Cycle Kinetics



Tumor-suppressor genes act primarily through their influence on the cell cycle. The life cycle of a cell that divides can be described by a four-phase cell cycle composed of M phase, G_1 phase, S phase, and G_2 phase. M phase is the phase of *mitosis*, when the cell physically divides. G_1 phase is the first *Gap phase* (or *Growth phase*), which occurs immediately after mitosis. During G_1 the cell is synthesizing proteins and growing to achieve the size, a normal cell has before splitting into two, during mitosis. S phase is a phase of *DNA Synthesis* (DNA replication) in preparation for cell division. The G_2 phase is characterized by DNA repair of errors introduced during DNA replication, and by preparation for the coming mitosis. Cells that cease dividing - or that rest for extended periods between divisions (such as liver cells) have temporarily or permanently exited the cell cycle by going into the G_0 phase at the end of the G_1 phase.

1.9 Terminology of Cell Death[6]

Cell death can occur by either of two distinct mechanisms, necrosis or apoptosis. In addition, certain chemical compounds and cells are said to be cytotoxic to the cell, that is, to cause its death.

The two mechanisms of cell death may briefly be defined as

- Necrosis
- Apoptosis

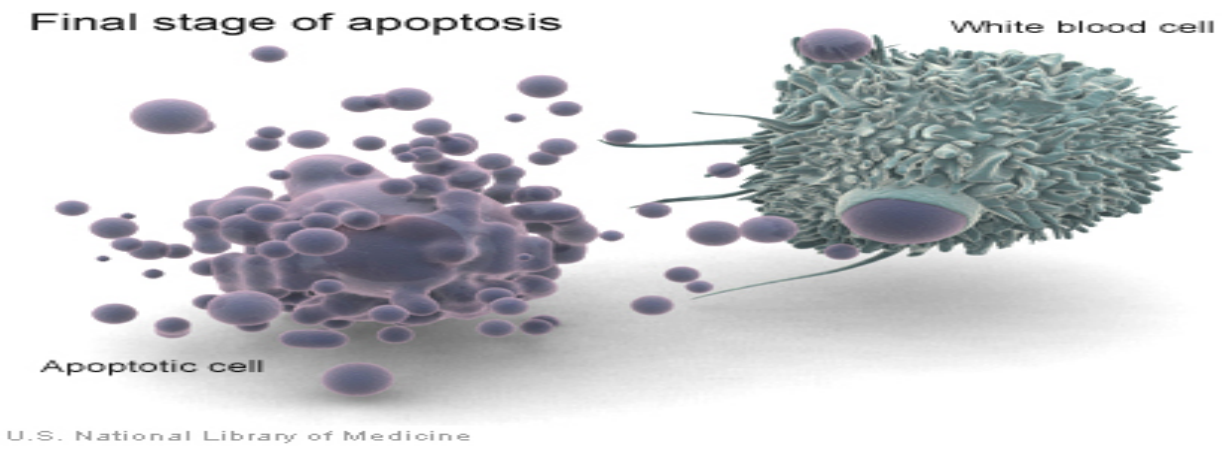
Necrosis

"Accidental" cell death is the pathological process which occurs when cells are exposed to a serious physical or chemical insult. Necrosis begins with impairment of the cell's ability to maintain homeostasis, leading to an influx of water and extracellular ions. Intracellular organelle, most notably the mitochondria, and the entire cell swell and rupture (cell lyses). Due to the ultimate break down of the plasma membrane, the cytoplasmic contents including lysosomal enzymes are released into extracellular fluid. Therefore *in vivo*, necrotic cell death is often associated with extensive tissue damage resulting in an inflammatory response.[6]

Apoptosis

"Normal" or "programmed" cell death is the physiological process by which unwanted or useless cells are eliminated during development and other normal biological processes.

Apoptosis or programmed cell death is a highly regulated pathway that is important in normal developmental processes as well as many diseases.[7] Apoptosis can be initiated by a variety of different stimuli that lead to a convergence of biochemical signaling pathways into a common collection of executioner molecules. Apoptosis is a mode of cell death that occurs under normal physiological conditions and the cell is an active participant in its own demise ("cellular suicide").



It is most often found during normal cell turnover and tissue homeostasis, embryogenesis, induction and maintenance of immune tolerance, development of the nervous system and endocrine-dependent tissue atrophy. Cells undergoing apoptosis show characteristic morphological and biochemical features. These features include chromatin aggregation, nuclear and cytoplasmic condensation, partition of cytoplasm and nucleus into membrane bound-vesicles (apoptotic bodies) which contain ribosomes, morphologically intact mitochondria and nuclear material. *In vivo,* these apoptotic bodies are rapidly recognized and phagocytized by either

macrophages or adjacent epithelial cells. Due to this efficient mechanism for the removal of apoptotic cells *in vivo* no inflammatory response is elicited.

1.10 Cancer Treatment

There are six main types of treatment for cancer and these are described below. It is fairly common for a combination of treatments to be used.

Active surveillance (or watchful waiting)

Some types of cancer grow very slowly and may cause no problems for many years. In this situation you may not need to have any treatment for some time, but your doctor will monitor you closely so that if the cancer does start to grow you can be given treatment at that time.

Surgery

An operation is done to remove the tumor. Surgery is often used if the cancer is only in one area of the body and has not spread. It may be used to remove lymph nodes if these are also affected by the cancer. It can sometimes be used to remove a cancer that has spread to another area of the body, but this is less common. The type of operation will depend on the area of the body affected by the cancer, and on the size and position of the tumor.

Radiotherapy

This is the use of high energy x-rays to destroy cancer cells, but cause as little harm as possible to normal cells. The radiotherapy is aimed at the affected area of the body and is very carefully

planned. It can cause side effects and the most common is tiredness. The side effects will depend on the part of the body that is being treated.

Chemotherapy

Chemotherapy is the use of anti-cancer (cytotoxic) drugs to destroy cancer cells. There are more than 50 different chemotherapy drugs. Some are given as tablets or capsules but most are given by drip (infusion) into a vein. The drugs go into the bloodstream and travel throughout the body to treat the cancer cells wherever they are. Sometimes just one chemotherapy drug is used, but often a combination of two, three or more drugs is given.

Chemotherapy can cause side effects. The side effects will depend on which drug (or combination of drugs) is used. There are now very good ways of preventing or reducing the side effects of chemotherapy.

Hormonal therapy

Hormonal therapies work by altering the levels of particular hormones in the body. Some cancers depend on certain hormones in order to divide and grow. By altering the level of hormones in the body, or blocking the hormones from attaching to the cancer cells the cancer can be controlled.

Drugs Used In Cancer Chemotherapy [10]

The main anticancer drugs can be divided into the following general categories

The mechanism of action of these drugs is discussed below they include:

- Alkylating agents and related compounds, which act by forming covalent bonds with DNA and thus impeding replication.
- Antimetabolites, which block or subvert one or more of the metabolic pathways involved in DNA synthesis.
- Cytotoxic antibiotics, which is substances of microbial origin that prevent mammalian cell division.
- Plant derivatives (vinca alkaloids, taxanes, campothecins) -most of these specifically affect microtubule function and hence the formation of the mitotic spindle.

Hormones, of which the most important are steroids, namely glucocorticoids, oestrogens and androgens, as well as drugs that suppress hormone secretion or antagonise hormone action.

Side Effects of Chemotherapy

Because the main target of chemotherapeutic drug is cell division, they will affect all rapidly dividing normal tissues, and thus they are likely to produce, to a greater or lesser extent, the following general toxic effects:

- Bone marrow toxicity (myelosuppression) with decreased leucocytes production and thus decreased resistance to infection
- Impaired wound healing
- Loss of hair (alopecia)
- Damage to gastrointestinal epithelium
- Depression of growth in children

- Sterility
- Teratogenicity.

1.11 Herbal Medicine

The term herb suggests something that is beneficial and has little potential for harm. We have been successfully using herb since ancient times.

Over the centuries, the use of herbs becomes more complex. The first herbal medicine was simply the roots, leaves and flowers of plants. Then in 19th century we began to study the plants and identify their constituents. Researchers discovered that some of theses isolated constituents were very powerful medicine. The evolution of herbs into drugs continued into the 20th century .Many wonderful and useful drugs emerged like antibiotics and powerful pain killers. Today people are discovering how well herbs work and have few side effects they have. As a result of this new interest herbal and pharmaceutical companies are isolating components of herbs. The herbal medicine is the most ancient form of health care known to human kind. Herbs have been used in all cultures throughout history.

Herbal Medicine and Cancer

Patients confronting a diagnosis of advanced (stage4) cancer face the statistical reality that conventional chemotherapy can affect a cure for only a tiny minority of all such cases. More often than not, the reasonable impulse of these patients to investigate alternative treatment options such as herbal medicine is met with negative responses from oncologists. As a result,

consumer-patients challenged with cancer seeking herbal medicine resources may resort to unreliable products promoted on the Internet or via multilevel marketing that offer dubious herbal "cures." Patients may also be less willing to disclose any use of these products to their physicians. Nurses as is often the case have the special challenge of being "between" both sides of this equation. The emerging integrative model of cancer treatment recognizes the importance of botanical medicine. However, despite extensive positive research data from experimental and preclinical studies, and the anecdotal clinical experience of many practitioners, patients, and cancer survivors, its potential in this field remains largely untapped and large scale clinical trials are generally unavailable. The reasons for this are multifactorial, and include historical, political, and cultural factors–and almost invariably a misunderstanding of the core principles of herbal medicine itself.

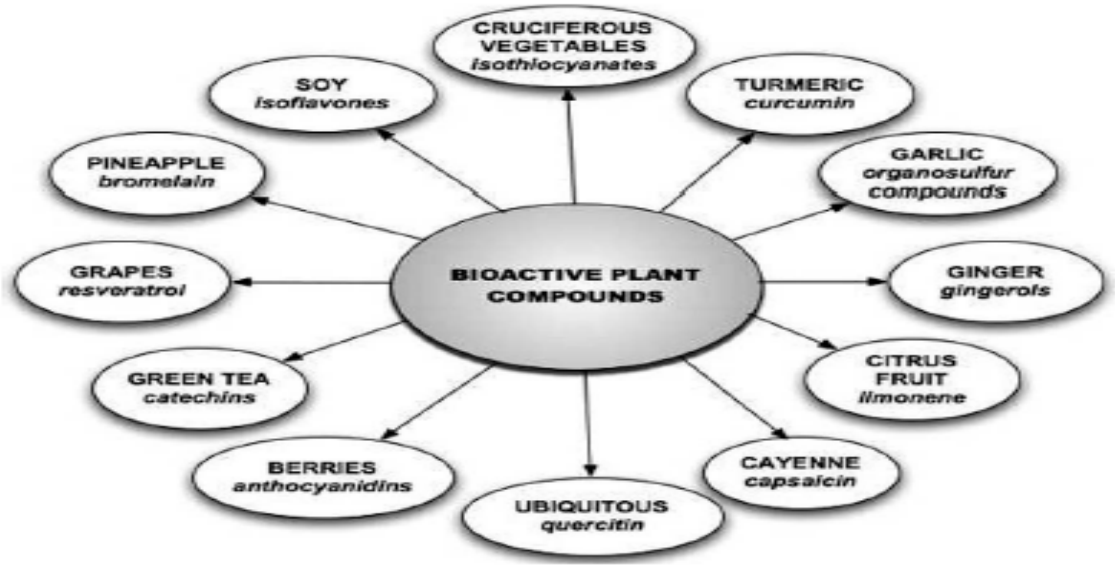
The use of herbs for medical benefit has played an important role in nearly every culture on earth. Herbal medicine was practiced by ancient cultures in Asia, Africa, Europe and the Americas. The recent popularity in use of herbals can be tied to the belief that herbs can provide some benefit over and above allopathic medicine and allow users to feel that they have some control in their choice of medications. The widespread use of herbs, either directly or as dietary supplements, has raised many scientific questions. Herbal products may act in a pathway similar to pharmaceuticals yet without side effects. Natural anti-inflammatory compounds abound in the herbal world and are found in green tea, the spices turmeric and rosemary, and others. Because the use of non steroidal anti-inflammatory drugs (NSAID) is associated with a reduced risk for

several cancers, it is at least plausible that natural NSAID should be explored for possible use as cancer preventives.

An important but often overlooked distinction exists between herbal *medicines*, (the practice) and herbal medicines (the plant-based remedies used in the practice of herbal medicine). The current trend of presenting so-called "scientific information" about herbs divorced from the context of herbal medicine that describes their medical use is rather like presuming that knowledge of needles will provide an understanding of acupuncture.

Chemopreventive agents are dietary ingredients which, being food derived, are considered pharmacologically safe. These ingredients contain bioactive molecules such as organosulfur compounds from garlic, polyphenols from green tea, and curcumin from turmeric, which exert the chemopreventive influences. For herbalists, the ultimate difference between food and medicine is basically one of intention. Herbalists have long used extractive techniques to concentrate the medicinal qualities of plants; by applying these time-tested methods to chemopreventive agents, these are transformed into therapeutic tools with anticancer activity. The same compounds, when intentionally deployed by practitioners as botanical medicine, transform prevention into treatment.[15]

There is a rapidly growing literature base on how plant-derived chemo preventive compounds have a potential role in the treatment of cancer.[16, 17] some of the more common chemo preventive compounds derived from dietary ingredients are listed below.



Chemopreventive plant compounds affect all phases of the cancer process (i.e. tumor initiation, promotion, and progression). Modern research is confirming that many chemo preventive dietary compounds are active at precisely the molecular targets that scientists seek to affect with the newer generation "targeted" biological response modifier drugs, typically the monoclonal antibody agents.

1.13 Plant Aloe Vera

History

The several thousand years' history of Aloe vera plant is a fascinating and captivating as a best seller historical novel. Long know for its legendry therapeutic properties, it was considered by some civilizations as the God. The curative virtues of the aloe are already known during ancient times. Authentic facts, testimonial and legendary narrations recount its history.

In the Bible, the spices is frequently cited as being used in herbal medicine since the beginning of the first century AD, because it is mentioned in the New Testament (John 19:39-40) *and there came also Nicodemus, which at the first came to Jesus by night and brought a mixture of myrrh and aloe.* Aloe vera also known as the medicinal plant is a species of succulent plant that probably originated in North America.

"If Aloe vera was to be discovered today, and its remarkable healing properties investigated, it would be hailed as the "wonder drug of this century". Aloe vera, on the other hand, has always been a natural product in whatever forms it has been used. Derived directly from the plant's leaves, Aloe vera is so effective in its natural state that no compelling reason has been found to spend the time and the enormous sums of money necessary to develop a synthetic version. It is hardly surprising then, that the wonder plant, Aloe vera, might spring from this tradition of using natural botanicals. Since 1979, Aloe vera has been the subject of increasing, vigorous, scientifically based investigations. Aloe vera (L) a number of families Lilliaceae is a popular perennial succulent plant.

Aloe vera is a semi tropical plant. There are over 250 species of Aloe grown around the world. It contains more than two hundred tonic ingredients including essential amino acids, minerals, vitamins, enzymes and steroids. Also contains the most essential components required by the human body. It is grown wild in hedge-rows in dry soil conditions and almost all parts of India. It can be grown even under constant drought conditions. Commercial cultivation and utilization of this plant with the application of technology can be of great value. Aloe vera is a succulent prickly plant of the Lily Family which grows in warm, frost-free climates and which has been known for centuries as a potent medicinal plant according the "folk medicines" of cultures around the world. Scientific and medical research teams have investigated Aloe in many countries but especially in the United States and Japan. Much of this research is of high quality, which underlines the considerable status that Aloe has attained as a herb with well proven medical attributes. At the same time one should call into question any claims for Aloe vera (or any other substance) for which there may be a lack of enough medical evidence.

1.14 The Elixir Reputation of Aloe

The complaints that Aloe has been shown to address, with varying degrees of scientific backing include: Acne, dermatitis, abrasions, boils, carbuncles, cuts, hair loss, headache, high blood pressure, indigestion, nausea, peptic ulcers, duodenal ulcers, colic, ulcerative colitis, gum sores, other mouth disease, pruritis, burns, AIDS, atherosclerosis and coronary heart disease cancers, diabetes, allergies, colds, parasites - e.g. protozoan infections, viral infections, infections generally, constipation, dandruff, oedema, chronic fatigue syndrome, genital herpes, gingivitis, haemorrhoids, herpes simplex and zoster, inflammation, insomnia, insect bites including bee stings, jelly-fish stings, menstrual cramps and period irregularity, radiation burns, rashes,

oesophagitis, sprains, seborrhoea, sunburn, tendonitis, leg ulcers, ulcerations generally, vaginitis, varicose veins, arthritis, gout, rheumatism, Candida infection, other fungal infections, dandruff, psoriasis, warts.

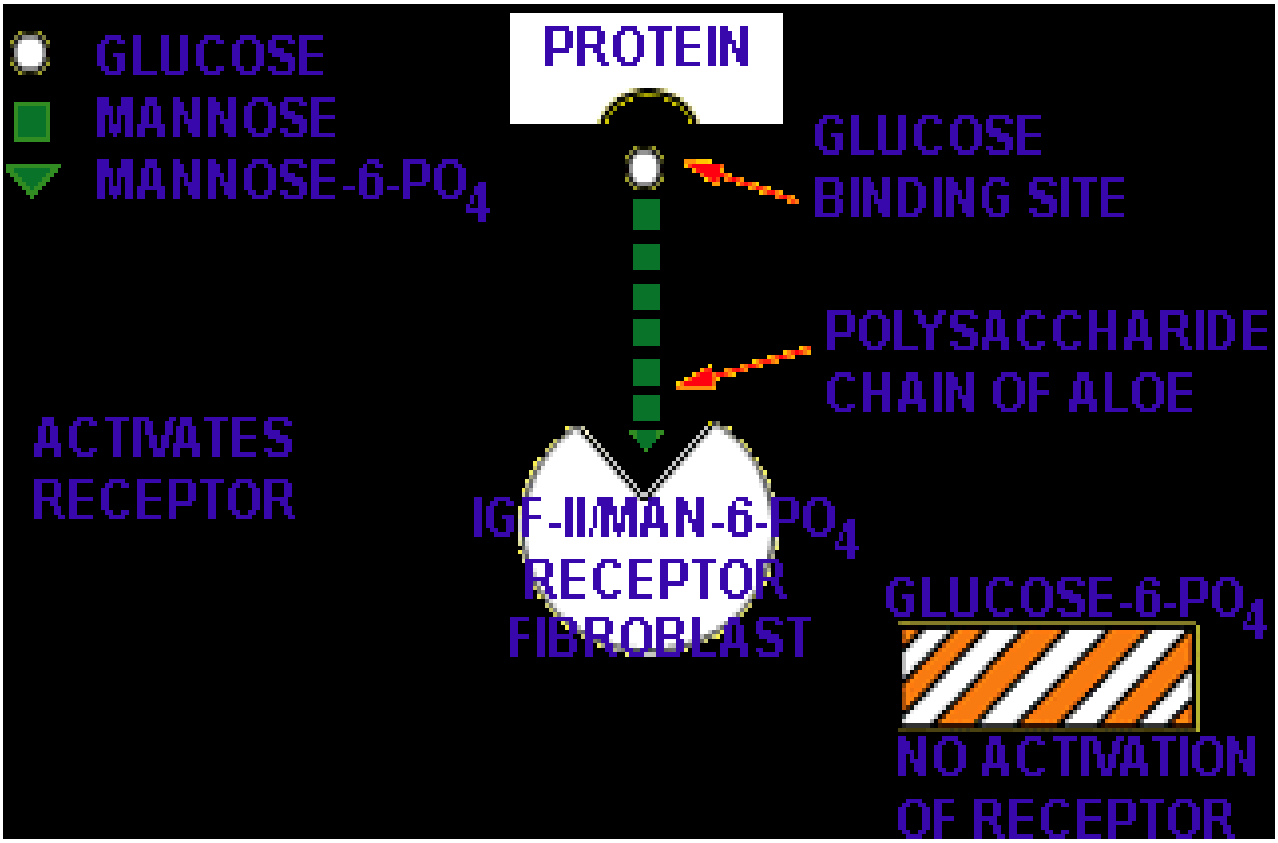
1.15 A Receptor for Aloe vera

It has been well established that the Insulin-like Growth Factor II (IGF-II) and mannose-6-phosphate (M-6-P) bind to the same receptor on the fibroblast. These two ligands bind at separate binding sites within the IGF-II/M-6-P receptor. However, the exact effect of these ligands binding to their individual binding sites is still unclear. One possible theory is that the binding of either ligand is capable of activating fibroblast proliferation. This would indicate that free M-6-P is a growth substance capable of yielding the same response as IGF-II. In Aloe, M-6-P is located at the end of the polysaccharide chain that fits into the receptor. This may be important in understanding how Aloe heals wounds and inhibits inflammation. It has been demonstrated that M-6-P improves wound healing in a straight line, dose-response fashion when compared with controls. For example, Glucose-6-phosphate,

When used as a control, does not activate the receptor in the fibroblast. The binding site for this glucose is at the other end of the polysaccharide chain of Aloe and is probably a weak to moderate covalent bond. Lack of response by this control indicates that M-6-P is specific to the receptor. This may be important in understanding how Aloe heals wounds and inhibits inflammation.

The receptor dynamics are part of the overall Aloe vera conductor-orchestra theory. One exciting characteristic of this theory is that the conductor-orchestra unit has little or no toxicity at very high gm/kg doses. Most of us working with Aloe vera have recorded no toxicity. In fact, we

feel that Aloe vera can modulate, reduce and may even eliminate toxicity of other biological agents.



1.16 Aloe vera is a Biological Vehicle

Compounds that are poorly absorbed through the stratum corneum of the skin need a vehicle to aid them in penetration. Glucocorticoids and vitamin C are not absorbed well and, as a result, most of the material is wasted when applied topically. A vehicle must be found that solubilizes steroids and Vitamin C while at the same time hydrates the stratum corneum. As

previously mentioned. Aloe vera's antiinflammatory; wound healing and analgesic properties make Aloe a "biological vehicle" that helps to nullify the detrimental activity of glucocorticoids but at the same time aids the penetration. Similarly, vitamin C is not readily absorbed by the tissue. However, vitamin C has been shown to help in the synthesis of collagen, since it may counterbalance the collagen breakdown to reduce the development of autoantibodies. In certain diseases, such as rheumatoid arthritis, the levels of vitamin C are low. Our data, thus far, suggests that Aloe vera aids in the absorption of vitamin C and adds to its biological activity. Although all the factors that control the absorption of vitamin C are not know, previous studies show esterifying ascorbic acid increases the availability to tissues so that the therapeutic dose can be reduced. In a similar manner, Aloe vera's activity as a biological vehicle to increase the absorption of vitamin C may be just as possible.

Aloe vera can solubilize water soluble compounds as well as lipid soluble substances. Also, it can hydrate the stratum corneum cell membranes to aid a range of materials in penetrating the skin. The biological activity of Aloe can add and even synergize with many agents in producing therapeutic effects so that we call Aloe vera a "biological vehicle."

1.17 Cold versus Heat Processing Methods

Data suggest that the time interval between leaf harvesting and processing (sun exposure) should be minimized (less than 24 hrs); heat exposure during processing should be minimized, especially if high temperatures are maintained for an hour or more. The whole leaf method can produce an Aloe extract which is high in total solids, high in retained high dalton (molecular weight) polysaccharides and glycosides with their scientifically demonstrated benefits,

Advantages of the Whole Leaf Cold Processing Methods

Advantages of the whole leaf, cold-processed, approaches include the following:

1. Maximizes the yield of desirable constituents.

2. Increases cost effectiveness of processing.

3. Increased total solids concentration.

4. Increased polysaccharide concentrations.

5. Virtual absence of undesirable anthraquinones.

6. Improved desirable characteristics for cosmetic usage.

7. Increased concentration in permeability factors increasing transdermal penetration.

8. Improved taste palatability.

9. Increased concentration of growth factors responsible for accelerating of healing.

10. Increased concentration of factors responsible for the stimulation of the immune system.

1.19 Immune System

There are several mechanisms which contribute to the immunological protection enjoyed by normal persons. Among these mechanisms the ingestion of bacteria and other potentially harmful agents by certain white blood cells (a process termed phagocytosis) and the formation of antibodies (formed by another group of white cells, the beta-lymphocytes) is probably the most

important. Scientific evidence suggests that Aloe gel contains substances which are active both in stimulating phagocytosis as well as stimulating the formation of antibodies.

In one study, the Aloe fractions were shown to increase phagocytosis when injected into guinea pigs. In another study, mice were injected intraperitoneally with Escherichia coli, which caused a serious infection to develop in the abdominal cavity, namely, peritonitis. Injects of materials from two species of Aloe (Aloe barteri and Aloe ferox) both stimulated phagocytic activity in the animals. It was demonstrated that phagocytic activity was depressed in adult patients with bronchial asthma. A mixture of amino acids derived from Aloe enhanced the depressed phagocytic function of the white blood cells in these asthma patients. In an additional study when certain materials (lectins) purified from Aloe were added to human lymphocytes raised in tissue cultures, the human white cells were stimulated to produce antibodies.

Perhaps the most remarkable studies concern the effect of Aloe fractions on the status of patients with HIV which causes AIDS. The polysaccharide fraction of Aloe was shown to exhibit antiviral activity and enhances cell function. The polysaccharide was given orally, 250 milligrams four times a day, to 8 patients with ARC (AIDS Related Complex), with Walter Reed staging from 3 to 6. Eight of eight patients showed improvement within 90 days of therapy with an average reduction of 2 Walter Reed stages. Fever and night sweats were eliminated in all patients; diarrhea was alleviated in two of three patients, and opportunistic infections (which are usually responsible for the death of the AIDS patient) were controlled or eliminated in six of eight patients. Two patients, unemployable because of the intensity of their symptoms, returned to full employment. Three of three patients showed a decline in HIV core antigen (P-24).

Initially positive HIV cultures became negative in three patients. Clinical toxicity and side-effects were entirely absent. Acute toxicity studies in animals showed no toxicity whatever at dosages 100 times those used in the pilot human experiments. These experiments however, were uncontrolled, and additional studies, utilizing appropriate scientific study design would need to be done before the data would be acceptable to the scientific community.

In plasma there are four interacting systems which serve vital protective functions. These include the following:

1. Intrinsic coagulation (blood clotting).

2. Plasminogen(prevention and dissolving of intravascular clots).

3. Kinninogen (inflammation).

4. Complement (destruction of intravascular bacteria).

The latter system, the complement system, consists of a series of proteins which require activation. When activated these proteins interact sequentially - a cascade phenomenon - and form circular, doughnut-shaped proteins, which are inserted into the surface membranes of bacteria, literally causing “holes” which permit the interior of the bacterium to become exposed to the environment, causing the death of the organism. Normally this complement system is stimulated by the presence of polysaccharides on the surface of the invading organism. Studies have shown that the polysaccharides (glucomannans) of Aloe can perform this function.

3. REVIEW OF LITRATURE

Larry N. Tumlinson[17] ***et al*** **(1985),** Study the methods and apparatus for extraction of aloe vera gel .the harvested leaves of aloe vera plants are positioned between a pair of endless moving belts for passing by a plurality of crushing rollers arranged in desired pattern.the crused leaves and extruded gel is than deposited on a drain grate to enable gravity flow separation the gel from the crused leaves. the drainage grate is sloped in order that the leaves will slowly moves across the drain grate to enable separation the gel while removing the leave from the gel collection area prior to the flowing of the contaminate aloin from the leaves .

Beatriz Varquez[13] ***et al*** **(1996)**, Studied the antiinflamatory activity of extract from alore vera gel, also studied the effect of aquous, chloroform and ethanol extracts of aloe vera gel on carrageenan induced edema in the rat paw and neutrophil migration into the peritoneal cavity stimulated by carrageenan. It also given the statement the capacity of the aquous extract to onhibit the cyclooxygenase activity. The anti inflammatory agents indomethacin and dexamethasone also decreased carrageenan edema and nutrophil migration. In the chloroform extract the chemical test performed by sterol type and anthraquinones were positive, these reault demonstrated that extract of aloe vera gel have an anti inflammatory action on the arachidonic acid pathways via cyclooxygenase.

Gowri chandrakasan[5] ***et al*** **(1996)** studied the influence of aloe vera on the healing of dermal wounds in diabetic rats. Full thickness excision/ incision wounds were geated on the back of rates and treated either by topical application on the wound surface or by oral adminisation of aloe vera gel to the rats. Full thickness excision / incision wounds were greated on the back of rates and treated either by topical application on the wound surface or by oral administration of aloe vera gel to therate

the result indiciate that aloe vera treatment of wounds in diabetic rates may enhance the process ofwound healing by influencing phase such as inflammation , fibroplasia ete.

Rob Fletcher[12] ***et al*** **(1997)** The analysis of the papers on Aloe Vera plant mention over time was completed by using agricda database 1970-1996.the study represents the reference listed below are from the biological abstract 1988-2000, and courtesy of silver platter information and more information is available from web http;/www.silverplatter.com.

H.Avila[6] ***et al*** **(1997)** studied cytotoxicity of a low molecular Weight fraction from aloe vera gel. Cytotoxicity of low moleqular fraction of aloe vera jel was determined by two different assays. Firstly exposure of monolayer of chicken fibroblast to induced LMWF to induced disruption of intracellular junctions and detachment of individual cell from bottom of the flask, with formation of cell free gaps in the monolayer. Secondly LMWF inhibited the production of reactive oxygen species by human polymorphonuclear leukocyte stimulated by Zymosan. This result conforms that aloe vera gel contains toxic low molecular wt. compounds.

C.T. Ramachand[15] ***et al*** **(1997)**, Studied the processing of aloe vera leaf gel: A review proper scientific investigation on aloe vera have gained more attaintion over the last decade due to reputable, medicinal pharmaceutical and food property. Present processing technique aims at producing best quality aloe vera product but end aloe product contains very little or virtually no active ingredient. Hence appropriate processing techniques should be employed during processing in order to extend the use of aloe vera gel.

Nobuycki Okamiura[21] ***et al*** **(1997),** Three new chromone components were isolated from the gel of Aloe leaf. The structures of these compounds, which we have named

8-C-glucosyl-(S)-aloesol, 8-C-glucosyl-7-0-methylaloediol and isorabai-chromone, were shown to be 8-C-8-D-glucopyranosyl-2[(S)-2-hydroxy]propyl-7-hydroxy-5-

methylchromone(8Cglucosyl(S)aloesol),8CDglucopyranosyl2(1',2'dihydroxylpropyl 7methoxy5methylchromone(8Cglucosyl7Omethylaloediol)and8CBD[2'0(E3caffeoyl] glucopyranosyl-2-[(~-2-hydroxy]propyl-7-methoxy-%methylchromone,respectively (isorabaichromone), by spectroscopic methods.

T. Reynolds[23] *et al* (1999).This review has largely upheld the therapeutic claims made in the earlier papers and indeed extended them into other areas. Treatment of inflammation is still the key effect for most type of healing but it is now realized that this is a complex process and that many of its constituent processes may be addressed in different ways by different gel constituent. Several reputable suppliers produce a stabilized aloe gel for use as itself or in formulations and there may be moves towards isolating and eventually providing verified active ingredients in dosable quantities.

Ni; Yawei[22] *et al* (1999), The inventions disclosed here in relate to composition for the sustained release of pharmacological agent compricing aloe pectins having the combination of both a high molecular weight and a low degree of methoxylation .aloe pectin having at least one of the following properties; degree of methylation of less than about 50% by moles; rhamnose content of from about 2 to about 50% by mole ;3-0-methyl rhamnose content of from about 0.1 to 5% by mole and capable of forming a gel in the presence of solution of a calcium salt .the aloe pectin are isolated from the leaves of a aloe by extraction ,where in the extraction is a accomplished by the supercritical fluid, water soluble organic solvent ,an acid ,an alkali, a chelating agent ,a bacteria,an enzyme and or combination thereof . according to the present invention ,pectins from gel and rind cell wall fibers of aloe vera extracted ,isolated and identified aloe pectins from pulp exhibit an off – white powder colour and produced clear solution when dissolved in water.

Kathi J. Kemper[11] *et al* (1999) Studied details about Aloe Vera plant and its proposed uses were topical treatment of burns, abration and canker sore ; laxative affect etc, and there other proposed use were experimental treatment of ulcers, cancer and HIV; immunostimulant. They state that aloe first gained popularity in united state in1930's with in the reports of its sources in treating X-ray burn and it has been boosted by claims that it has boosted by clams that it has been similar anti-aging effect to vitamin A derivative. Biochemistry of aloe also has been studied which gives details about chemical constituent in plant.

Teresa Pecere[16] *et al* (2000), Studied the aloe- emodine is new type of anticancer agent with selective activity against nuroectodermal tumors. Aloe- emodine is a hydroxianthraqunone present in aloe vera leaves, has a specific in vitro and in vivo anti nuroectodermal tumor activity. The growth of human neuroectodermal tumor is inhibited in mice with severe combined immunodeficiency without any appreciable toxic effect on the animals. Takinig into a account its unique cytotoxicity profile and made of action, AE might represent a conceptually new lead anti-tumor drug.

Nathona Chaiyakunapruk[9] *et al* (2005), Studied the efficacy of aloe vera used for burn and wound healing: A systemic review. Aloe vera has been tradionally used for burn healing but clinical evidence remains unclear. Author conducted a systemic reiew to determine the efficiency of topical aloe vera for the treatment of burn wound; four studies with the total of 371 patients were included in this report based on the Meta analysis using duration of wound healing as an outcome measure. Well designed trial with sufficient details of the contents of aloe vera product should be carried out to determine the effectiveness of aloe vera.

Ayse Can[1] *et al* (2004), Studied the effect of aloe vera leaf gel and pulp extract on the liver in type 2 diabetic rate models. Diabetes rate were separated into four groups and each group was given by 15 d PBS solution pulp extract, leaf gel extract, glibendamide liver tissue were examined histologically and it was conducted that

aloe gel extract has a protective effect compare to glibendamide against hepetotoxicity produced by diabetes if used in the treatment type 2 diabetes.

Christian Rabe[10] *et al* (2005) Studied acute hepatitis induced by an aloe vera preparation: A case report. Aloe vera plant extract is widely used in phytomedicine. The first cause of the acute hepatitis due to this compound was described. He concludes that Hepatitis in a57 year old female could be linked to the ingestion of aloe vera compound. The patient's hepatitis resolved completely after discounting this by medicines.

Taiwo V.O[19] *et al* (2005), Consumption of Aqueous Extract of Raw *Aloe Vera* Leaves:Histopathological and Biochemical Studies in Rat and Tilapia. Forty-five juvenile tilapia and 30 weanling albino rats exposed to water containing 50, 100 and 150ppm of aqueous extract of *Aloe vera* leaves for 96 hours and 28 days, respectively were used for this study. Fifteen tilapia and 10 rats exposed to clean water (0 ppm *A. vera*) served as controls. Clinical signs, mortality, gross and histologic organ pathology in the tilapia; weekly haematology, plasma biochemical parameters and organ pathology were monitored in the rats. Fish cultured in water containing *A. vera* exhibited erratic swimming patterns, rapid opercular movements, skin depigmentation and died within 24-96 hours. Gross and histologic tissue lesions in the test fish include skin depigmentation, pale and shriveled gills, dull, opaque and sunken eyes, stunting and clubbing of gill filaments, vacuolar degeneration and necrosis of gill epithelial cells, hyaline degeneration and necrosis of myofibrils, calcification of vasa vasori, hepatocellular vacuolar degeneration and necrosis. Haematologic and plasma biochemical changes in test rats include moderate to severe normocytic normochromic anaemia, hypoproteinaemia, increased AST levels, and decreased cholesterol and triglyceride levels. Gross and histologic tissue lesions include mild to moderate pulmonary congestion, flabbiness of the heart, hepatomegaly, mottling of kidneys, vacuolar degeneration and necrosis of hepatocytes, Kupffer cell hyperplasia,

periportal fibrosis, glomerular and tubular degeneration and necrosis, matting and clubbing of small intestinal villi, catarrhal enteritis and goblet cell hyperplasia. The severity of these changes increased with increasing concentrations of *A. vera*. No mortality, gross or histologic changes were observed in both control fish and rats. Results from this study show that consumption of water containing extracts of raw *A. vera* is very toxic to fish and rats. The serious health implication for human consumption of raw *A. vera* is discussed.

Katrin Lachenmeier[20] *et al* (2005) Studied the quality controle of aloe vera beverages .commercial material was reported to be frequently adulterated by artificial preservative or to lack significant amount of aloe ingredients .HPTLC and HS-SPME /GC/MS methods to acceses the authenticity of aloe vera beverages were developed in this study, aloin to differentiate between authentic and adulterated products .the HS-SPME method employed in this work allowed to detect the preservatives of benzic acid ,sorbic acid and pHB-esters .in 17 of 24 (71%) currently available of aloe food products an illegal addition of preservatives of up to 1000 mg /L could be ascertained but rather legal concerns ; controles have to be intensified to ensure the sufficient product quality with regards to preservatives.

Etim OZ[4] *et al* (2006) Studied the protective effect of aloe vera juie on lindane induced hepatotoxicity and cytotoxicity. Serum levels of hepatic enzyme markers. Glutamate pyruvate transamine glutamate oxaloacetate transaminase gama glutamyl transferase and alkaline phosphatase were determined after oral adminisation of aloevera leaves extract and lindane .the bend of polychromatic erythrocytes was also observed pretreatment with aloe vera leaves extract at concentration of 1.0 ml/ kg body weight significantly decresed the serum level.

P. Dehdari[7] *et al* (2006) Studied and demonstrated details about the aloe vera as semifinish product like gel, powder and finish product like health drinks, also its cultivation ,benefit of aloe vera in medicine, its biochemistry, processing of plant into

gel and powder, its also gives commercial information, economical and world market report and its recommendation.

Rana P. Singh[8] *et al* (2006), Studied the mechanism of action of novel agent of prostate cancer chemoprevention. Despite the advantage in the understanding of Pca growth and development, it is still the leading cause of mortality due to cancer in men. In vivo preclinical study has indicated chemo preventive effect of many agents in Pca xenograft and transgenic mouse models. This review focuses on novel Pca chemo preventive observation in laboratory studies which could provide the rationale for the prospective use of chemo preventive agent.

Rajendran A.[2] *et al* (2007) Studied and demonstrated the separation and characterization of the phenolic anthraquinones from aloe vera. Among the different from of A.vera sap contains more phenolic anthraquinones of 7.09 mg/g of lyophiligate than the remaining from all the three form of aloe and chromatographed fraction of aloe sap in five different solvent mixtures were characterized by IR, Flurescence spectroscopy, Mass spectroscopy and U.V.specroscopy.

Keivan Zandi[3] *et al* (2007) An article entitled antiviral activity of aloe vera against herpes simplex virus type 2: An in vitro study.herpes simplex virus type 2 is an enveloped virus which causes genitel herpes and some other complication finding a new natural anti –HSV-2 drug has been a subject of interest for scientist because of severe side effect and a development of some resistance mutants of this virus regarding the several enviormental factors affecting the chemical and biological composition of aloe vera ,it is tried to evaluate anti- HSV-2 activity of this plant.

Mike Adamas[14] *et al* (2007), Studied and given details about the aloe vera as miracle: a natural medicine for cancer cholesterol dibetes, inflammation, IBS and other health conditions. It involves details about aloe vera boost immune function and

destroys cancer tumors, aloe vera halts inflammation,aloe vera inhances skin health, stabilizes blood sugar in dibetis, it also lowers cholesterol and triglycerides, also relives joint and muscle pain, amplifies the anti oxidant effect of vitamins and aloe vera heals radiation burns from radiation cancer treatments.

R. M. Coopoosamy[18] *et al* (2007), Studied the Traditional use, antibacterial activity and antifungal activity of crude extract of *Aloe excelsa.* The fleshy leaves and roots of most species within the *Aloe* family are used in many traditional treatments (Mabberley, 1987). Traditional healers and indigenous people utilize mainly the leaf sap of this genus widely for the treatment of wounds, burns, rashes, itches, cracked lips and cracked skin (Cera et al., 1980). Antimicrobial activities on the crude extract of *Aloe exclesa* was carried out in attempts to validate the use by traditional healers in the use of there latex and gel exudates for various medicinal ailments.

6. List of materials and instruments

Methanol	Qualigens fine chemicals, Mumbai
PHA	Gibco Invitrogen Corporation
Giemsa stain	Qualigens fine chemicals, Mumbai
Fetal calf serum	Gibco, Invitrogen Corporation
Eagles MEM Media	Gibco, Invitrogen Corporation
Doxorubicin HCl	United Biotech Pvt Ltd, Bagbania
Docetaxel trihydrate	Dr Reddys Lab Ltd
Potassium chloride	Rankem, New Delhi
Acetic acid	Merck Ltd, Mumbai
Ethanol	Bangal Chemicals and Pharmaceuticals Ltd, Kolkata
Normal saline	Claris Life Science Germany Ltd, Mumbai
Phosphate buffer	Merck Ltd, Mumbai
Centrifuge	Remi, Mumbai
Water bath	Remi, Mumbai

Incubator	Remi, Mumbai
Cyclomixer	Remi, Mumbai
Balance	Sartorius, Japan
Trinocular microscope (Flourescent – CH201)	Olympus, Japan
Laminar air flow	SM. Scientific Instrument Pvt. Ltd., Delhi
CO_2 incubator	Hera cells, Heraeus
Inverted microscope (CKX41)	Olympus, Japan

7. METHODOLOGY

7.1 Sample Collection

Plant *Aloe vera* was collected from cultivated area on well fertilized soil and healthy, matured plant has been selected for studies. Plant has selected from Tindal, Erode district, and Perumanallur, Coimbatore district.

7.2 Sample Identification

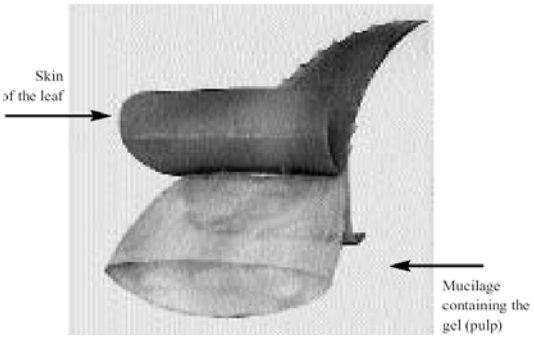
Specimen of *aloe vera (L.)* was harvested and collected plant has been identified by Botanical survey of India at Coimbatore. Fresh leaves of the cultivated plant were used in these studies.

7.3 Processing of Sample

Harvesting- the healthy and matured *Aloe vera* sample plant has been selected from the farming land. Maturity of the plant should be noticed.

7.4 Washing and cutting

The harvested aloe vera plant's leaves has been cutted from the base in to single single leaf, then leaves were washed under running tab water 2-3 times again with distilled water to remove dust particle from leaf surface, and finally with anti microbial solution, 60% alcohol to remove surface pathogens from the leaves .Then the washed leaves were processed for cutting. To cut and separate out the gel and leaf lining, outer layer sharp, sterile knife has been used[17].



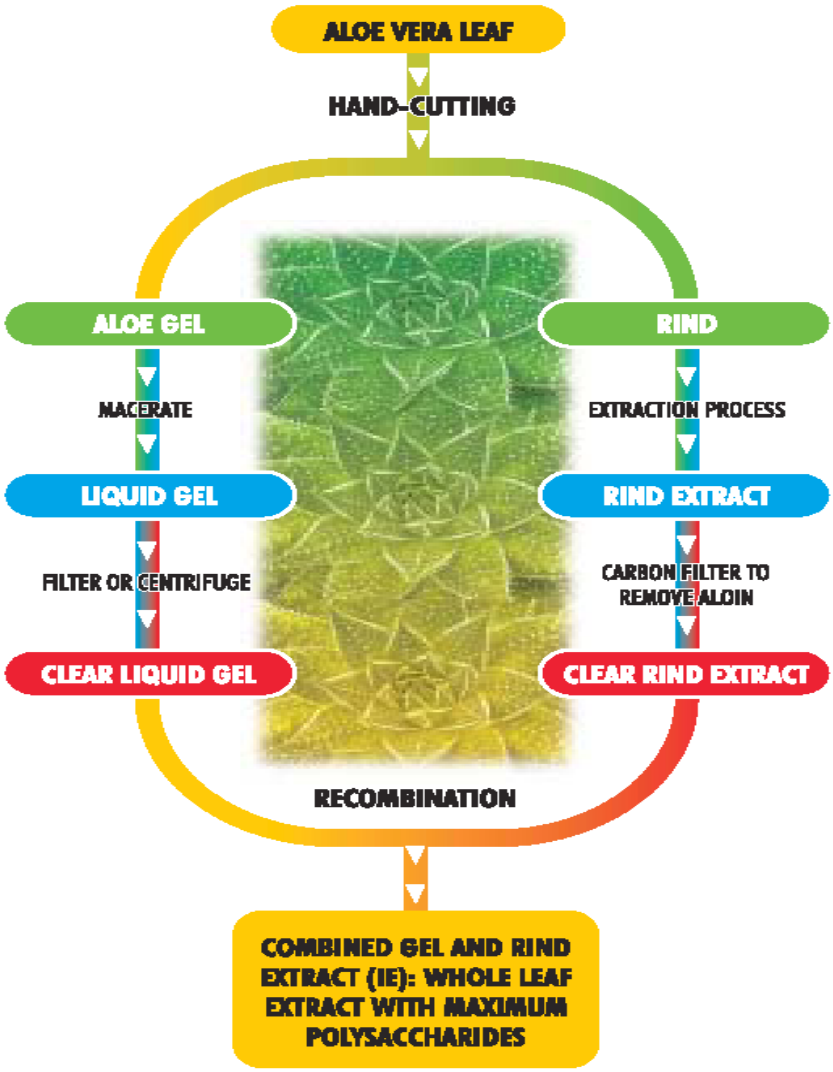
Then the gel and skin of the leaf has been cutted in to small pieces in required quantity for the extraction.

7.5 Extraction[17]

Extraction of *Aloe vera:*

A. vera leaves (big and healthy leaves) weighed, washed and cut in the middle, the gel were separated by scratching with a spoon. ***Aloe vera* leaves pulp extract:** the leaves pulp was cut in to the small pieces (514 gm) and homogenized with phosphate buffered saline solution (PBS; pH 7; 600 ml) by means of a blender. The extract was

kept at 4º C overnight, and then filtered through muslin cloth and the filtrate Centrifuged at 20000 rpm for 30 min. at 2 º C in a refrigerated centrifuged. The green pallet was discarded and the clear yellow supernatant was taken and lyophilized. thus 10 gm of Aloe vera leaves pulp extract was obtained .the extract used in the experiment was (7.5%) prepared by dissolving the powder in PBS and mixing it thoroughly via magnetic stirrer.



***Aloe vera* leaf gel extract:**

The gel was homogenized in a blender, then diluted with an equal volume of PBS and homogenized for a second time and the extract was kept at 4º C overnight then filtered through cloth. The clear filtrate was kept and lyophilized at -20 ºC

.

7.6 Lyophilization:

In the process of lyophilization, extract are subjected to extreme dehydration in the frozen state and than sealed in a vacuum. In this condition, desiccated (lyophilization) extract of plants contain its constituents viable for many years. The clear liquid filtrate after an overnight was lyophilized in deep refrigerator at -20 ºC. So there is a direct sublimation from liquid to solid state. And finally aloe vera was obtained in powder form. Which is further underpass by phytochemical tests to conform its constituents present or not.

7.7 Preliminary Phytochemical tests:

A. General test:

1. Boil (100mg) of drug with 100 ml of distilled water and than add little amount of kieslghur and than filtered.

from the above filtered solution (5ml) add 0.2 gm borex and heat until dissolved, few drops of liquid into test tube nearly full of water was poured on it, green fluorescence appears.

Bromine test: add 2 ml of aloe solution into 2 ml of freshly prepared bromine solution. It gives pale yellow precipitate.

B. Specific test:

1. Nitric acid test:

Add 5 ml of aloe solution in to 2 ml of nitric acid, which gives brownish color, rapidly change green for cape aloe and deep brownish red color will change into green for aloe baridensis.

2. Nitrous acid test;

2 ml of aqueous solution of aloe, added into few small crystal of sodium nitrate and add little acetic acid, which gives red pink to caramine color (Barbados) and lesser pink by cape aloe.

C. Klunge's Isobarbaloin test:

Add 20 ml aqueous solution of aloe into drops of saturated copper sulfate solution followed by 1 gm of sodium chloride and 10 ml of alcohol (90%), which gives wine red color and at least persist for ½ hours. In case of case aloe lesser coloration of wine-red color which rapidly feds to yellow color appears.

D. Modified Bronstanger's test:

In 1 ml of the sample add few drops of ferric chloride and dilute Hcl were added which bring oxidative hydrolysis followed by CCl_4, rose pink to cherry red color appears when the solution is shaken with dilute NH_3.

7.8 Experimental work

***In vitro* cytotoxic activity**

7.8.1 Cell culture[21]:

Materials: To a 500 ml bottle of EMEM media, add 55.5 ml IFS (Inactivated fetal calf serum) to a final concentration of 10% and 5 ml Penicillin- Streptomycin to a final concentration of 1µg/ml strep.

Serum Media: Add 10 ml Glutamine upon opening to 1 lt.

T 75 flasks and pipettes.

70% ethanol in a spray bottle.

The Cell:

Melanoma and fibro sarcoma 2 cell line that grows very quickly and is easily maintained. They are grown in EMEM media with fetal calf serum and antibiotics and maintained at 37°C or lower. Stocks will be grown in T75 flasks and will be split about every 3 day. Experiment will be done in 6 well plates.

Sterile Technique:

All cell culture were must be performed in the tissue culture hood, also known as biological safety cabinet. The hood protect from the aerosol contamination by filtering all

of airs that enters. In addition, to prevent other contamination, spray down all the inside working surfaces of the hood with 70% ethanol and wipe the surface dry with wipe before and after using the hood. Set all the materials need in the hood before bringing in the cells such as the media and flasks, but also try not to clutter the hood with unnecessary items. In general work is to be done quickly with good sterile technique to avoid contamination.

Procedure:

Cell Culture for Melanoma cell.

The B16F10 melanoma cell line was kindly provided by A.R. Lab Salem. Cells were cultured in Eagle's minimum essential medium (EMEM), supplemented with 10% fetal calf serum (FCS) and streptomycin plus penicillin (100µg/ml and 100 IU/ml, respectively).Cells were cultured in a 5% CO_2 humidified atmosphere at 37°C until near confluence. All the processes were carried out in a vertical laminar flow chamber.

Cell Culture for Sarcoma cell:

The SW982 fibro Sarcoma cell line was kindly provided by A.R. Lab Salem. Cells were cultured in Eagle's minimum essential medium (EMEM), supplemented with 10% fetal calf serum

(FCS) and streptomycin plus penicillin (100µg/ml and 100 IU/ml, respectively).Cells were cultured in a 5% CO_2 humidified atmosphere at 37°C until near confluence. All the processes were carried out in a vertical laminar flow chamber.

Procedure:

Volume: Start culture at 25 million cells in 12 ml media in T75 flask.

Duration: culture was grown for 3 days and than split.

1. Tap the flasks to loosen cells from the surface. If cells are not resuspended well by tapping, remove cells with scraper. These cells are not very adherent, so they will lift off the surface fairly easily.
2. Pipette up and down 10X to create a cell suspension.
3. Count of cells with a hemocytometer.
4. Resuspend cells to appropriate density and seed in flasks.

7.9 Drug treatment:

B16F10 Melanoma cell:

Aloe vera extracts were prepared in increasing final concentrations, ranging from 25 to 200µg/ml. The drug extracts were treated with plates containing confluent monolayer of B16F10 melanoma cell lines. Negative control group was melanoma cell line only, and the positive control group was treated with doxorubicin (0.032µg/ml).

After incubating for 24 hrs at 37˚C, the cells were trypsinized (0.25% in PBS) and then centrifuged at 1000 rpm for 5 min, washed twice with fresh medium, and resuspended with fresh medium. Cell viability was counted for each concentrations of crude extract as well as for control.

SW982 fibro sarcoma cell:

Aloe vera extracts were prepared in increasing final concentrations, ranging from 25 to 250µg/ml. The drug extracts were treated with plates containing confluent monolayer of Sarcoma cell lines. Negative control group was sarcoma cell line only, and the positive control group was treated with doxorubicin (0.032µg/ml). After incubating for 24 h at 37˚C, the cells were trypsinized (0.25% in PBS) and then centrifuged at 1000 rpm for 5 min, washed twice with fresh medium, and resuspended with fresh medium. Cell viability was counted for each concentrations of crude extract as well as for control.

Aloe vera sample drug dilutions:

For **melanoma** cell line extract has been made from ranges 25 to 200 µg/ml as followed by dilution procedure –
100 mg aloe extract dissolved in 100 ml of PBS solution to produce 1000 µg/ml, from this solution different dilution has been made as shown below

- 25µg/ml, 0. 25 ml solution dissolved in 10 ml of stock solution.
- 50 µg/ml, 0.5 ml solution dissolved in 10 ml of stock solution.
- 100 µg/ml, 1 ml solution dissolved in 10 ml of stock solution.
- 150 µg/ml, 1.5 ml solution dissolved in 10 ml of stock solution.
- 200 µg/ml, 2 ml solution dissolved in 10 ml of stock solution.

For **Sarcoma** cell line extract has been made from ranges 25 to 200µg/ml as followed by dilution procedure –

100 mg aloe extract dissolved in 100 ml of PBS solution to produce 1000 µg/ml, from this solution different dilution has been made as shown below

- 25 µg/ml, 0. 25 ml solution dissolved in 10 ml of stock solution.
- 50 µg/ml, 0.5 ml solution dissolved in 10 ml of stock solution.
- 100 µg/ml, 1 ml solution dissolved in 10 ml of stock solution.
- 150 µg/ml, 1.5 ml solution dissolved in 10 ml of stock solution.
- 200 µg/ml, 2 ml solution dissolved in 10 ml of stock solution.
- 250 µg/ml, 2.5 ml solution dissolved in 10 ml of stock solution.

Control Groups:

1. Positive control was treated with standard doxorubicin (0.032 µg/ml)/
2. Negative control was only cancer cell lines.

7.10 Trypan blue exclusion assay: growth and viability test[21].

Material:

Hemocytometers

Trypan blue diluted in required quantity with PBS. Store at room temperature.

Hand held counters.

Principle: - It is based on the principle that live cells possess intact cell membranes that exclude certain dyes, such as Trypan blue, Eosin or Propidium whereas dead cells do not.

Procedure:

1. Prepare a uniform cell suspension as described below.

Suggestion: Dilute cells 1:5 or 1:10 before counting. Dilute in media and take an initial aliquot of cells. (Smaller aliquot may be less uniform).

2. Mix a small aliquot of cells 1:1 with trypan blue solution. (NOTE) - When counting cells for normal pass aging, trypan blue is not needed. Viable cells excluded trypan blue, while dead cell stain blue due to uptake of dye.

3. Center a cover glass over the hemocytometer chambers. Fill one chamber with the cells using a Pasteur pipette. The solution will be entering the chamber by capillary action. Do not over fill. If the cell will spread in two lateral grooves adjoining the grid table, clean the hemocytometer and repeat the application.

4. With an inverted microscope, count the cells located in to four corners and the center square of the hemocytometer, for a total of five squares. Using visualization of the cells. Use a hand held counter to record the number of cells counted. During the trypan blue exclusion assay don't worry about calculating the percentage of viability of the cells. Simply generate total live cell count by only counting the circular white cells and not the blue cells. The total cell count should exceed 100 to be reliable. If after counting five squares, 100 cells have not yet counted then count additional squares.

5. The percentage of viable cells can be calculated by using the formula shown below.

6. Wash cells off the hemocytometer with water. Spray some 70% EtOH and let dry.

To evaluate growth and viability of the treated and untreated cells, the percentage of viable and non-viable cells was determined, using trypan blue exclusivity stain. Cell growth and viability was measured by adding 0.4% trypan blue in 0.9% saline to a 50% dilution, and cells were counted using the hemocytometer. Briefly, 0.5 ml of the trypan blue solution was transferred to a test tube and 0.3 ml of PBS plus 0.2 ml of the trypsinized cell suspension (dilution factor of 4) were added. The final solution was thoroughly and gently mixed and allowed to stand for 5 min. Then a drop of this dye-cell Suspension was loaded on to both chambers of the haemocytometer. Cells were examined and counted in duplicates under light microscope at 100 X (Olympus, Japan). Percentage cell viability was calculated by the formula:

$$\text{Cell viability} = \frac{\text{No. of viable cells (unstained cells)} \times 100}{\text{Total no of cells (Stained and unstained)}}$$

5. PLAN OF WORK

The present work was carried out to study *in vitro* cytotoxic activity of *Aloe vera* extract against B16F10 melanoma and SW982 sarcoma cell line.

The following experimental protocol was designed to allow a systemic approach to study.

Plant Processing:

- Plant sample collection.
- Processing of sample.
- Extraction.
- Phytochemical investigations

***In vitro* Cytotoxicity activity.**

- Procurement of cancer cell line.
- Cell culture.
- Trypan blue exclusion essay for cell viability.

2. PLANT PROFILE

2.1 HISTORY

As is often the case with so called 'miracle plants' their exaggerated reputation actually discredits them. Aloe vera is a truly wonderful plant with no shortage of members for its fan club. It has a very long and well established reputation as a healing plant, particularly for skin conditions, minor cuts and abrasions. The dried latex, which is not the same as the gel, but instead derives from the yellow juice contained in the pericyclic tubules of the inner leaf, is a well known laxative.

Despite the fact that Aloe has been in documented use for at least 3500 years, controversial and contradictory information about this plant abounds. The earliest reference to its use can be found in the famous Egyptian Ebers Papyrus, which dates back to 1500 BC and is widely regarded as one of the earliest documents on what was to become the western Materia Medica. However, it is more than likely that it has been commonly used for centuries before it was recorded. In fact, it seems more likely that Aloe was such a commonly used plant, that earlier documents (of which few have survived) never even bothered to mention it. In the hot and dry countries of the Mediterranean and the Middle East Aloe vera served as a soothing household remedy for sunburns and moisturizing cosmetic lotion.

More efficient processing methods have recently been developed that utilize the whole leaf and just remove the green parts of the leaf in a cold process involving a cellulose dissolving substance. This retains the biochemical activity of the Aloe vera Leaf in its integrity. The resulting gel is of a yellow color as it still retains the aloin, which is the bitter, laxative compound. Another whole leaf extraction method involves the same cold process leaf processing in the first step, but then utilizes short duration low temperature controlled sterilization techniques that kill off bacteria without the addition of chemicals. The resulting gel is then concentrated in a vacuum chamber and subsequently dehydrated into a water soluble compound that retains the biochemical

activity indefinitely without the addition of any preservatives. This method is currently regarded as the most efficient method, even though heat is used in the process.

2.2 TAXONOMICAL FEATURE

Kingdom	-	Plantae
Division	-	Magnoliophyta
Class	-	Lilliopsida
Order	-	Asparagales
Family	-	Lliliaceae
Genus	-	Aloe
Species	-	*Aloe vera.*

2.3 COMMON NAME

Bengali and sans.	-	Ghritakumari
Hindi	-	Ghee – kanwar
Kannad	-	Kolasoare
Telagu	-	Kalabanda
Tamil	-	Kathalai

2.4 SYNONYMES

Aloe barbedensis mill, Aloe indica royle , Aloe perfoliata L, Aloe vulgaris Lim, Indian Aloe, First Add Plant, Burn Aloe, Miracle plant.

2.5 BIOLOGICAL SOURCE

Is Stemless and very short succulant plant, 60-100 cm tall, spreading by offsets. Aloe vera growing as ornamental plant. Aloe vera plant belongs to family Liliaceae.

2.6 MACROSCOPIC CHARCTERSTIC

Color

Color peagreen, (when young spotted with white); bright yellow tubular flowers

General appearance

The gel is a viscous, colorless, transparent liquid.

Organoleptic properties

Viscous, colorless, odourless, taste slightly bitter.

Shape and Shape

Leaves 30, 50 cm long and 10 cm broad at the base, bright yellow tubular flowers 25, 35 cm in length arranged in a slender loose spike; stamens frequently project beyond the perianth tube.

2.7 CHEMICAL CONSTITUENTS

From the gel

Polysaccharide: glucomannan and acemannan.

Other : carboxy peptidase, Mg,Zn,Ca,glucose,cholesterol,salicylic acid,prostaglandin precursor,vitamin A,C,E, Saponins,amino acid.

From leaf lining:

Anthraquinone glycosides: aloin,aloe-emodin,barbaloin

2.8 Experimental Studies

Aloe vera: Potential Clinical Benefits

1. Cardiovascular: none

2. Pulmonary: none

3. Renal and electrolyte balance: none

4. Gastrointestinal/hepatic: Stimulant laxative (leaf lining); gastric and duodenal ulcers (gel); inflammatory bowel disease (gel, experimental use)

5. Neuropsychiatric: none

6. Endocrine: Hypoglycemic (gel)

7. Hematologic: none

8. Rheumatologic: none

9. Reproductive: Emmenagogue (leaf lining, traditional use)

10. Immune modulation: Immunostimulant, anti-inflammatory (gel)

11. Antimicrobial: Antibacterial, antiviral, antifungal (gel)

12. Antineoplastic: Antitumor, attenuation of adverse effects of cancer therapies (gel)

13. Antioxidant: none

14. Skin and mucus membranes: Vulnerary (wound healing), psoriasis remedy (gel)

15. Other/miscellaneous: none

2.9 PLANT PHOTOES

Cultivation on the farming soil.

Plant with flowers.

8. Results and Discussion

8.1 Processing of plant:

The plant has collected and identified as Aloe barbidensis.

8.2 Extraction:

The extraction has done successfully by comparatively study of extraction procedure. Finally 765 mg Aloe extract was extracted with PBS solution by cold processing method. Liquid extract was than lyophilized at -20^{o}c to get fine solid powder extract of Aloe vera.

8.3 Phytochemical investigation:

Lyophilized extract was than brought into phytochemical tests find to out its polysaccharides and glycosides constituents present or not in final extracted extract, which exhibits cytotoxicity to cancerous cells.

This investigation exhibits that lyophilized extract retains its cytotoxic constituents in it.

The present study was carried out to evaluate cytotoxic activity of *Aloe vera* leaves extract on the B16F10 melanoma cancer cell line and SW982 fibro sarcoma cell lines.

8.4 Cytotoxic activity:

Cytotoxic activity of above *Aloe vera* extracts was evaluated on the basis of “Trypan blue exclusion assay” for cell viability.

8.5 Cell viability:

Cell viability can be defined as the number of healthy cells in a sample. The most straight forward method for determining viable cell number is a direct counting of the cells in a haemocytometer.

Melanoma cell-To evaluate the cell viability of B16F10 melanoma cell line treated with aloe vera extract of the drug *Aloe vera*, groups were taken which are as follows:

1. Control group –In this group out of 100 total cells 95±1.33 viable and 05±1.33 non viable cells were found.
2. Chemotherapy drug treated group - In this group out of 100 total cells 20±1.22 viable and 80±1.22 non viable cells were found.
3. *Aloe Vera* extract treated group – This group is consisted of following subgroups:

i) 25µg/ml extracts treatment - Out of 100 total cells 52±1.66 viable and 48±1.66 non viable cells were found.

ii) 50µg/ml extract treatment - Out of 100 total cells 46±2.03 viable and 54±2.03 non viable cell were found.

iii) 100µg/ml extracts treatment - Out of 100 total cells 38±1.39 viable and 62±1.39 non viable cells were found.

iv) 150µg/ml extracts treatment - Out of 100 total cells 29±2.11 viable and 71±2.11non viable cells were found.

v) 200µg/ml extracts treatment - Out of 100 total cells 22±1.95 viable and 78±1.95 non viable cells were found.

Treatment of *Aloe vera* extracts against B16F10 melanoma cell line, in all concentration range showed decrease in percent cell viability, as compared to that of negative when examined by "Trypan blue exclusion assay". In overall variation of test samples, *Aloe vera* extract showed its best activity in the conc. of 200µg/ml, which was approximately equal to the activity of standard drug doxorubicin. Therefore, we concluded from cell viability assay that extract with phosphate buffer exhibited very good cytotoxic activity as compared to that of positive control. Results are shown in Table no. 1.

Sarcoma cell - To evaluate the cell viability of SW982 fibro sarcoma cell lines. Treated with aloe vera extract of the drug *Aloe vera*, groups were taken which are as follows:

1 Control group –In this group out of 100 total cells 96±1.21 viable and 04±1.21 non viable cells were found.

2 Chemotherapy drug treated group - In this group out of 100 total cells 21±1.01 viable and 79±1.01 non viable cells were found.

3 *Aloe Vera* extract treated group – This group is consisted of following subgroups:

i) 25µg/ml extracts treatment - Out of 100 total cells 50±1.31 viable and 50±1.31 non viable cells were found.

ii) 50µg/ml extract treatment - Out of 100 total cells 44±1.12 viable and 56±1.12 non viable cell were found.

iii) 100µg/ml extracts treatment - Out of 100 total cells 39±1.93 viable and 61±1.93 non viable cells were found.

iv) 150µg/ml extracts treatment - Out of 100 total cells 34±1.20 viable and 66±1.20non viable cells were found.

v) 200µg/ml extracts treatment - Out of 100 total cells 30±2.01 viable and 70±2.01non viable cells were found.

vi) 250µg/ml extracts treatment - Out of 100 total cells 27±1.01 viable and 73±1.01 non viable cells were found.

Treatment of *Aloe vera* extracts against, SW982 fibro sarcoma cell lines in all concentration range showed decrease in percent cell viability, as compared to that of negative when examined by "Trypan blue exclusion assay". In overall variation of test samples, *Aloe vera* extract showed its best activity in the conc. of 250 µg/ml, which was approximately equal to the activity of standard drug doxorubicin. Therefore, we concluded from cell viability assay that aloe vera extract with phosphate buffer exhibited very good cytotoxic activity as compared to that of positive control. Results are shown in Table no. 2.

8.2 Table No.1

Effect of different concentration of *Aloe vera* extract on B16F10 melanoma cancer cell viability test-

Drug conc. (µg/ml)	Total cells	Viable cells	Nonviable cells
Control	100	95±1.33	5±1.33
25	100	52±1.66	48±1.66
50	100	46±2.03	54±2.03
100	100	38±1.39	62±1.39
150	100	29±2.11	71±2.11
200	100	22±1.95	78±1.95
Doxorubicin(0.032)	100	20±1.22	80±1.22

8.3 Table 2:

Effect of different concentration of *Aloe vera* extract on SW982 fibrosarcoma cancer cell viability test-

Drug conc. (µg/ml)	Total cells	Viable cells	Nonviable cells
Control	100	96±1.21	4±1.21
25	100	50±1.31	50±1.31
50	100	44±1.12	56±1.12.
100	100	39±1.93	61±1.93
150	100	34±1.20	66±1.20
200	100	30±2.01	70±2.01
250	100	27±1.01	73±1.01
Doxorubicin(0.032)	100	21±1.01	79±1.01

Table-3.

Preliminary phytochemical tests:

Chemical test name	Result
A. General test Bromine test- Nitric acid test- Nitrous acid test-	 + ve + ve - ve
B. Klunge's isobarbaloin test-	- ve
C. Modified bronstanger's test-	+ ve

Fig No. 1-Effect of different conc. of *Aloe vera* on percent cell viability

Concentration Vs Percent viability

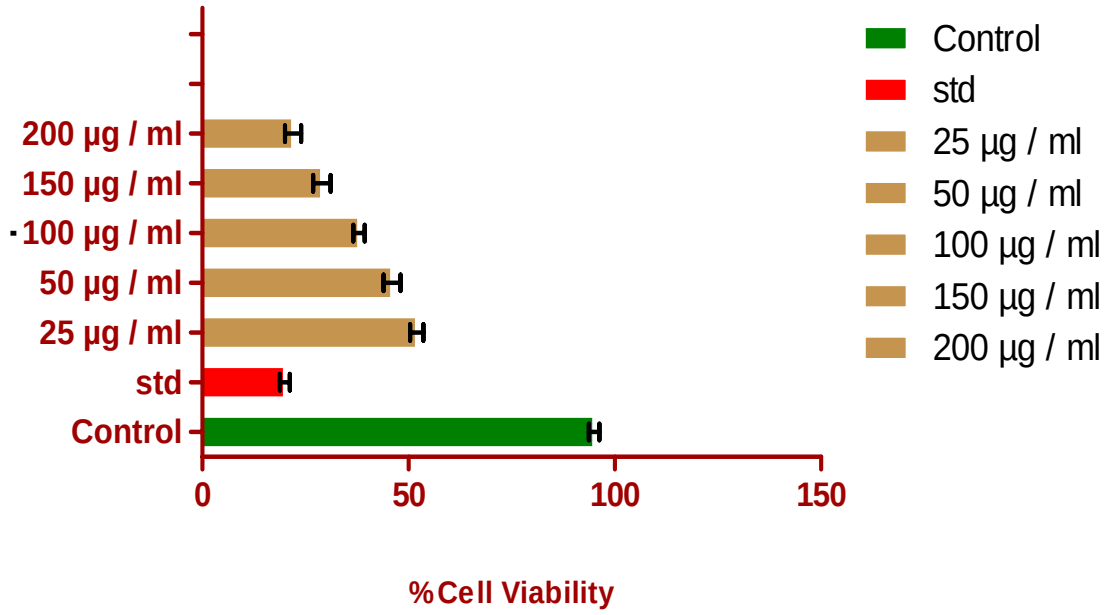


Fig.No.-2. Effect of aloe vera on difference concentration on SW980 Fibrosarcoma cell.

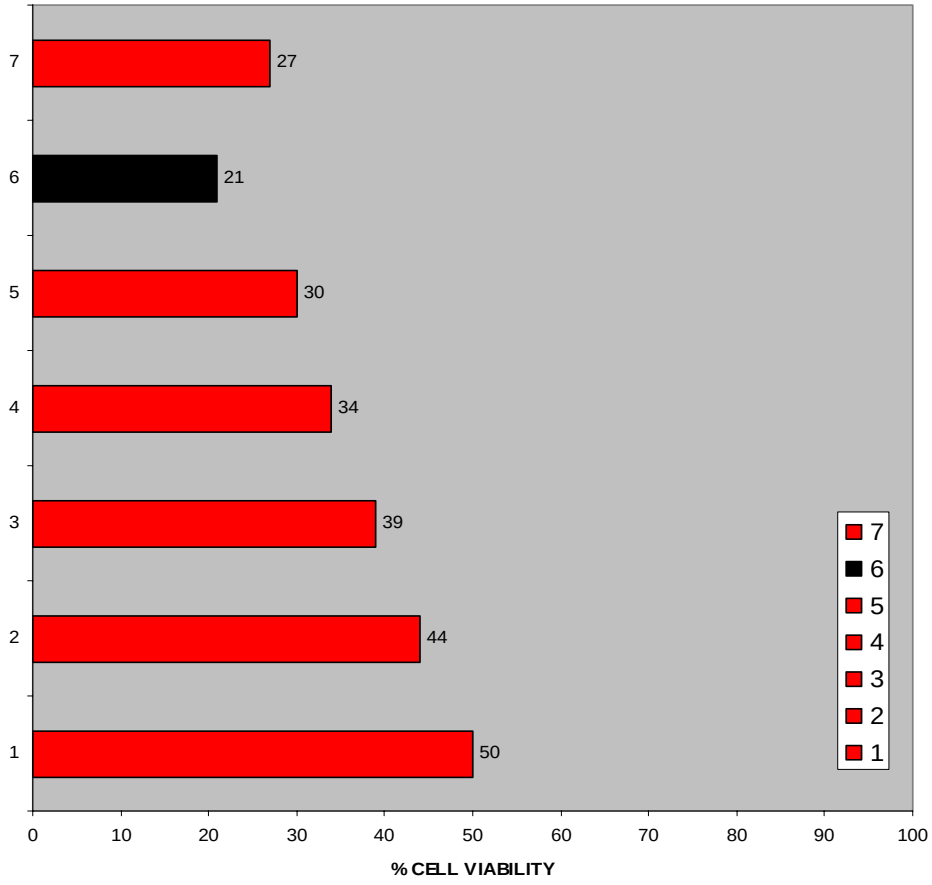
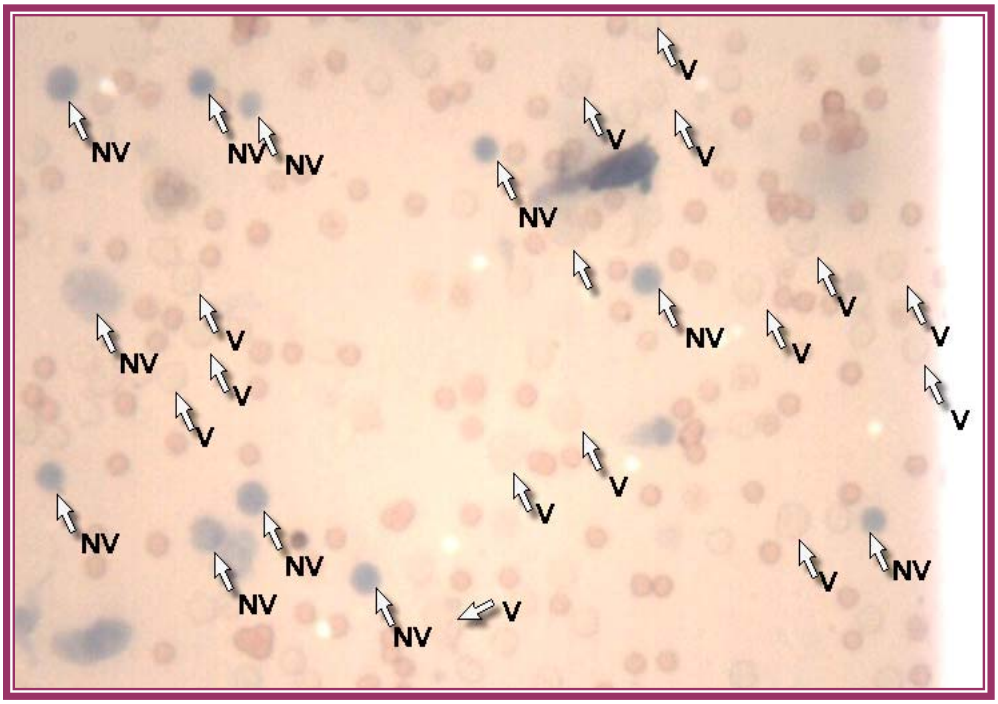


Photo - Showing "Trypan blue exclusion by non viable cells"



9. Summary and Conclusion

Cancer is one of the most dangerous and dreadful disease of the 20th century and spreading further with continuance and increasing incidence in 21st century. An alternative solution to western medicine embodied with severe side effect is the use of medicinal plant preparation to arrest the disease.

This piece of work is mechanized to prove potentiality of herbal extracts of *Aloe vera* to kill the cancerous cells and to protect the normal cells from the cytotoxic effect of anticancer drugs. Here the culture is being performed for B16F10 melanoma and SW982 fibrosarcoma cancer cell, the molecular involvement and potency of chemotherapeutic drug in cancer cell is well presented.

In vitro cytotoxic activity performed revealed that *Aloe vera* exhibited good cytotoxicity. The best cytotoxic activity by *Aloe vera* was shown at 200µg/ml concentration for B16F10 melanoma cell line and 250µg/ml for SW982 fibro sarcoma cancer cell as compared to that of standard anticancer drug.

In future this piece of work can be mechanized for purification and identification of active constituents present in *Aloe vera* by using various analytical techniques like thin layer chromatography, column chromatography, FT-IR, mass spectroscopy etc. Also, this work can be extended for *in vivo* study of antitumor activity by measuring tumor volume and also for *in vivo* cytoprotection activity. The statistical correlation can be established

on the above based information, so that this wonderful plant of *Aloe vera* which is well known for its antispasmodic activity and used since long as “cosmetics product”, can be used as a drug of choice during cancer chemotherapy, which is available at low cost and is poor man friendly. In near future such products will be a boon to man kind for cancer and HIV patients.

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